PB-216 720

THE DEVELOPMENT OF METHODS FOR USING BIOASSAYS IN THE CONTROL OF PULP MILL WASTE DISPOSAL

Charles E. Warren, et al

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

1957

DISTRIBUTED BY:

NTIS

National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 216 720

THE DEVELOPMENT OF METHODS FOR USING BIOASSAYS IN THE CONTROL OF PULP MILL WASTE DISPOSAL[1]

Charles E. Warren
Department of Fish and Game Management
Oregon State College

and

Peter Doudoroff [2]
Robert A. Taft Sanitary Engineering Center
U. S. Public Health Service

LIBRARY
SANITARY ENGINEERING CENTER
CINCINNATI, OHIO

The control of discharges of industrial wastes adequate for the protection of other uses of receiving waters usually is a complex problem, and the regulation of pulp mill waste discharges with their potentials for dissolved oxygen depletion, toxic action, and the production of bacterial periphyton, commonly referred to as "slime", certainly is no exception. Waste disposal regulations intended for wide application are difficult to formulate, because differences of effluent and receiving water characteristics make each discharge problem unique, necessitating individual consideration. Certain minimum dissolved oxygen concentrations for receiving waters have been included in the water quality standards established by various regulatory bodies. Routine determination of the dissolved oxygen concentration in the receiving water is a convenient check on the adequacy of effluent controls for the prevention of excessive deoxygenation of the water; and the biochemical oxygen demand determination, when properly used, may be a reasonably good measure of the oxygen depleting potential of a

---

1. Miscellaneous Paper No. , Oregon Agricultural Experiment Station.

2. Stationed at Oregon State College, Corvallis, Oregon.

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

particular waste, suitable for predicting some effects of pulp mill waste discharges. But similarly adequate restrictions and measurements necessary for preventing damage to receiving water uses through toxic action or "slime" production are not now generally available. The lack of specific regulations having to do with toxicity results from a lack of knowledge as to permissible levels of particular toxicants in receiving waters, as well as a lack of means of determining the concentrations of some of the toxicants in these waters. The acute toxicity bioassay has been proposed as a measure of the relative toxicity of effluents, but satisfactory means of application of the results of the bioassays to waste control problems have not yet been fully developed nor widely used. While examination of a receiving stream will indicate the amount of "slime" on the bottom, little is known of the amounts of "slime" which will not interfere with at least some of the stream uses. Means of determining by bioassay the "slime" producing potentialities of particular wastes are now being investigated, but methods of applying the measurements to waste discharge control have received little consideration. Methods being developed at Oregon State College for utilization of various bioassay techniques for control of pulp mill waste disposal will be discussed in this paper.

Routine chemical analysis of kraft process wastes for certain known toxicants usually present has been undertaken at some mills, but such analysis does not now make possible adequate control of waste discharges. Different investigators have determined the tolerance of various aquatic organisms to the known individual toxic constituents of the wastes, such as sulfides, mercaptans, and the resin acids and their salts, (Cole, 1955; Ebeling, 1931, 1932; Haydu, Amberg and Dimick, 1952; Van Horn, Anderson and Katz, 1950). However the identified toxic compounds in kraft

process wastes for which the tolerances of aquatic organisms have been studied are certainly not the only toxic compounds present in these wastes, and there is evidence that they are not always even the most important ones. McHugh (1954) performed preliminary experiments indicating that the toxicity to fish of the effluent of one Northwestern kraft mill was not largely ascribable to any of the know toxic constituents of the waste. The toxicity appeared to be principally due to some unidentified substance or substances present in the stream-distillable, ether-soluble fraction of the waste.

Even when the toxicants present in a complex waste are known and chemical means of analysis are available, the toxicity of the waste cannot always be reliably predicted on the basis of toxicity tests of the individual constituents, because of possible antagonisms, synergisms, and other interactions of the various compounds, and because of differences in receiving waters. Thus, at the present time, chemical analysis of known toxic constituents of kraft process wastes cannot be generally used as a means of predicting the potential toxicity of these wastes to aquatic organisms .

Many biologists concerned with the problem of protecting valuable aquatic resources from damage by industrial effluents believe that the best means of evaluating the toxic potential of a complex waste usually is a toxicity bioassay of the waste itself. A bioassay of the entire waste, diluted with water from the receiving stream, certainly is the most direct, rapid, and reliable means of evaluating the acute toxicity of the waste to any organism in that particular water. However, most aquatic organisms will not be able to thrive indefinitely in their native environment at concentrations of a waste which they can tolerate for a short

period in an aquarium. Since only some fraction of the concentration tolerated by the organisms in the bioassay can be deemed permissible in a receiving water, "application factors" to be applied to the bioassay results, for determining the necessary dilution, must be developed. Hart, Doudoroff, and Greenbank (1945) proposed a formula by which the presumably safe or permissible concentration can be calculated from the results of a 48-hour bioassay with suitable fresh-water fish as test animals, through the use of "factors of safety". One factor of safety was "intended to make allowance for possible variations of tolerance of different organisms under varying conditions." Another factor of safety would adjust for possible difference between the concentration which an organism can tolerate for 48-hours and the concentration which this organism could tolerate indefinitely. The formula for the estimation of the permissible concentration of a waste and the safety factors employed in this formula were based on general considerations and experience. It was recognized that the formula and factors were necessarily arbitrary, and that refinements of the proposed procedure for determining permissible waste concentrations in receiving waters could only come about through intensive research. At the present time the formula initially proposed by Hart, Doudoroff, and Greenbank for general application is no longer considered by the authors to be satisfactory. It has become apparent that simpler application factors must be developed, each intended for use in connection with the control of discharges of one particular kind of waste only.

This paper is a discussion of research at Oregon State College in methods of determining permissible concentrations of pulp mill wastes in receiving waters by means of bioassays and application factors.[2]

---

2. This research is supported in part by the National Council for Stream Improvement, Inc.

Standard and convenient toxicity bioassay methods are being developed, and application factors for making use of the results of these bioassays are being determined experimentally in part by means of "artificial streams" approximating natural conditions. Pulp mill wastes, whose acute toxicity has already been evaluated by means of bioassays, are introduced continuously in varying amounts into these streams for periods as long as one month. A similar approach is being developed for the control of the discharge of pulp mill wastes so that resulting "slime" production can be maintained at biologically harmless levels. It is hoped that such an experimental approach will lead to the development of application factors which will make possible the realistic use of bioassay results in preventing damage to aquatic resources by pulp mill effluents.

STANDARDIZED TOXICITY BIOASSAYS

Most of the methods of chemical analysis used routinely for industrial waste control yield fairly reproducible results. Standardization of analytical methods and the availability of excellent "reagent grade" chemicals have contributed much to this reproducibility. Suggested standard methods for toxicity bioassays of industrial wastes recently proposed by Doudoroff et. al. (1951) are gaining wide acceptance. Yet, a standardized methodology will not alone make the results of toxicity bioassays highly reproducible. The reagent for any bioassay is the organism or association of organisms involved, and living organisms by their very nature are highly variable. Before the results of toxicity bioassays of wastes can become fairly reproducible, in addition to a standardized methodology, a generally available, convenient, and uniformly tolerant test organism must be found. That this organism may be

more tolerant or less tolerant of the waste being tested than are other organisms found in the receiving water need be of no particular concern if suitable application factors based on experience with a variety of organisms are to be developed, for as long as the toxic action on the different organisms is similar, their relative tolerances can probably be established.

The sensitivity of a fish to a particular toxicant might be expected to vary with its age, size, species, genetic constitution, and recent environmental history. Thus, the species of fish selected to serve as the standardized reagent for toxicity bioassays must be readily available at all times of the year wherever the tests are to be performed, and must be such that it can be bred in the laboratory and that individuals of uniform age, size, genetic strain, and environmental history can be produced. The common guppy, Lebistes reticulatus (Peters), appears to meet satisfactorily these requirements.

The guppy is generally available from tropical fish dealers throughout the United States and even the world. Guppies are live-bearers (i.e. ovoviviparous) and breed throughout the year. The female has a brood every four weeks or so, containing an average of about 45 young with the number ranging upward to as high as 187 (Axelrod and Schultz, 1955). Guppies require little care, and an abundance of literature is readily available on their rearing. While tropical fish fanciers frequently separate individual females in breeding tanks (to prevent other fish from eating the young and to make selective breeding possible), this procedure requires too much time and space to be practical for a busy waste-control or research laboratory. Good results have been obtained

using 50-gallon aquaria containing a dense growth of Ceratophyllum demersum, an aquatic plant which provides a place of hiding for the young guppies, thus assuring adequate survival. These aquaria are uniformly lighted with fluorescent lamps operated for controlled periods by means of time switches. The temperature of the water is thermostatically controlled at 25° Centigrade. Each aquarium contains 30 to 40 adult females and 10 to 15 adult males. The fish are fed a commercially prepared tropical fish food of good quality. The young fish are removed from the aquaria daily and acclimatized to the temperature of holding tanks, usually 20° Centigrade, this being the temperature at which the bioassays are to be performed. The fish are used when they reach an age of about one week, older fish being saved for future use as brood stock. The young guppy has many advantages as a test animal for toxicity bioassays in the busy laboratory . Fish of uniform size and age are available at all times of the year right in the laboratory. Their genetic and environmental histories and their diet are under complete control. They require little care, and three or four 50-gallon aquaria should furnish enough fish even for a laboratory performing bioassays continually. Finally, the small size of the young guppy makes possible the use of small vessels that require little space. Through the use of very small vessels even comparatively minute waste fractions can be tested successfully.

The method by which young guppies have been utilized in toxicity bioassays of kraft process pulp mill wastes has differed from the routine bioassay method proposed by Doudoroff et. al. (1951) only insofar as modifications have been necessary for best achieving the objectives of the particular waste discharge control procedures being developed. The

tests have been performed in a constant temperature room at a temperature of 20° C. Test containers have been ordinary one-gallon, wide-mouth, glass jars. Ten young guppies and three liters of test solutions have been placed in each jar, twenty fish in two jars usually having been used for testing each waste concentration. Twenty fish yield somewhat more precise results than the minimum ten fish suggested by Doudoroff et.al. While the minimum duration of tests proposed by these workers was 48 hours, the bioassays with the guppies have been of only 24-hour duration. The bioassay time has been thus shortened in order to make the technique somewhat more applicable where speed of toxicity determination is important. Since it is intended that the 24-hour bioassay results will be used only in conjunction with application factors derived from prolonged experiments, it is not necessary that the bioassays be continued for 48 hours if the relative toxicity of waste samples can be adequately assessed in 24 hours. Because kraft process wastes have some oxygen demand and the test dilutions may lose some of their toxicity on standing in open jars, the fish have been transferred to fresh test solutions after 12 hours, thus maintaining dissolved oxygen concentrations above 4 parts per million (auxiliary method C of Doudoroff et.al.). The 24-hour median tolerance limit, which is the concentration at which just 50 percent of the fish are able to survive for 24 hours, is estimated by straight-line graphical interpolation (on semi-logarithmic coordinate paper) as described by Doudoroff et.al. (1951).

ARTIFICIAL STREAMS FOR DETERMINING BIOASSAY APPLICATION FACTORS

By themselves, standardized toxicity bioassays of brief duration have limited practical value. Chronic pollutional injury to valuable

populations of aquatic organisms may occur much more often and may be more important than spectacular mortalities of fish and other animals caused by acutely harmful pollutional conditions of relatively brief duration, which may or may not have a lasting adverse effect on the populations. Even those environmental conditions which are not demonstrably fatal can have a thoroughly destructive affect on aquatic populations if they interfere with the reproduction, development, growth, normal activity, or migratory movements of the individual organisms, or destroy their supply of food. Accordingly, while the determination of the concentration of a toxic waste which can be tolerated for 24 hours by 50 percent of the young guppies is a necessary step in the use of bioassays for the control of waste discharges, much additional information is likewise necessary.

For each kind of toxic waste, the relationship between the 24-hour median tolerance limit for the guppy and the maximum concentration at which a variety of fishes and other important aquatic organisms can thrive indefinitely in their natural environment must be established. This relationship is not easily determined, for in no laboratory experiment can the complex natural environment be accurately modeled. Furthermore, it must be recognized that the manner of harmful action of a complex toxic waste on organisms is not always the same at rapidly fatal and at slowly fatal concentrations, nor is it the same in the natural environment as in an aquarium. Also the mode of action on different kinds of organisms under comparable conditions may not be the same. The harmful agent involved may not be the same in every case and the concentrations of the various harmful constituents of a complex waste can vary more or less

independently. Yet, it is only the establishment of some fairly definite relationship between bioassay results and the long-term effects of a waste on the biota of any receiving water that will make the standardized toxicity bioassay a dependably useful tool in the control of waste discharges. Much remains to be learned of the possible complex relationships considered above, with particular reference to pulp mill wastes, and not all of the problems can be solved through laboratory experimentation. However, encouraging results have been obtained when comparing bioassay results with the effects of kraft process pulp mill wastes on various aquatic organisms in "artificial streams" having certain selected features of the natural environment. These are to be described presently. Other applicable laboratory methods also have been developed for the study of the influence of water quality upon the development of fish, their food consumption and utilization, and their activity potential, or ability to resist currents (Warren and Doudoroff, 1957). Laboratory experiments on the avoidance reactions of salmonid fishes to kraft and sulfite process pulp mill wastes have been performed in order to obtain information concerning the possible influence of these wastes on the movements of fish in their native environment (Jones et al., 1956).

Each artificial stream used for the development of bioassay application factors consists of two wooden troughs 10 feet long, 10 inches wide, and 10 inches deep, containing stream bottom materials and river water which is renewed continually at the rate of about one and one-half liters per minute. The water in one of these troughs can be rapidly circulated by means of a centrifugal pump, to create a riffle-like current. The desired amount of pulp mill waste is added continually to the river

water entering this trough by means of a chemical pump. From this trough the water overflows into the second trough, which resembles a pool with little current. Six such artificial streams are now in use for testing simultaneously a series of different waste concentrations. Into these artificial streams are placed various fresh-water organisms, including fish, insect larvae, crustaceans and molluscs. Proper lighting makes possible the growth of various microscopic plants attached to the bottom materials. Thus, the influence of a particular waste concentration on a wide variety of stream organisms under conditions similar to those found in their natural environment can be studied.

Several batches, or samples, of kraft process pulp mill waste are required for completing a single experiment lasting as long as one month. These samples may differ in their toxicity. The 24-hour median tolerance limit (for young guppies) of each waste sample is determined by bioassay, and a concentration which is always some fixed fraction of this value is maintained in each artificial stream throughout an experiment. For example, when the media tolerance limit of a waste sample is 4.0 percent by volume, concentrations equal to 0.025, 0.05, 0.1, 0.2, and 0.4 times this TL_m value (or 0.1, 0.2, 0.4, 0.8, and 1.6 percent waste by volume) are maintained in five of the artificial streams (the sixth receiving no waste and serving as a control) until a fresh sample of waste must be used. If the new sample is found to be twice as toxic, the 24-hour median tolerance limit for guppies being 2.0 percent, the waste concentrations in all streams are adjusted accordingly, ranging from 0.025 x 2.0% to 0.4 x 2.0% (or from 0.05 to 0.8 percent by volume). The largest fractional factor by which the 24-hour median tolerance limit for guppies has been multiplied,

to arrive at waste concentrations at which no harm to any of the organisms can be demonstrated, is regarded as a tentative or provisional bioassay application factor. Before any such conclusion can be reached, the experiment must be repeated many times, however, with a large variety of organisms, which are counted at the beginning and end of each experiment to determine whether or not any unusual mortality has occurred.

It has not yet been demonstrated that the observed injury to organisms at the lowest demonstrably harmful waste concentrations tested in the artificial streams and the acute toxicity of the waste to guppies are referable to the same constituent or property of the waste. If it can be shown that different waste components are responsible for these effects and that their concentrations in kraft pulp mill effluents can vary independently, the tentative toxicity bioassay application factors arrived at in the above-described manner may be at least partially invalidated. Determination of the cause of pollutional damage to various organisms even in artificial streams is difficult. Kraft mill wastes are toxic to aquatic animals, but they also cause the growth of "slime" which can blanket the bottom materials, eliminating or seriously modifying the habitat niches of insect larvae and other organisms. The oxygen demand of the waste can result in a deficiency or complete lack of dissolved oxygen beneath the blanket of "slime", even when there is abundant oxygen in the water above. Sometimes insects themselves become covered with "slime", which may further interfere with their respiration. These various effects of the wastes can be observed and closely studied in artificial streams, and some of the factors can be independently controlled in seeking to establish the cause of death of the experimental

animal.. For example, oxygen gas can be introduced into the water flowing into the streams, thus maintaining relatively high dissolved oxygen concentrations, and non-toxic organic matter can be added to produce or increase "slime" growths. Comparatively simple experiments on the toxicity of the wastes to the various organisms, the dissolved oxygen requirements of these organisms under varying conditions, and so forth, can be performed also without the use of artificial streams, and some of these tests already have been undertaken. The results of such tests can help to interpret the observations on artificial streams. Tests with artificial streams are nevertheless deemed necessary. Some organisms do not live well in captivity unless they are held under conditions closely resembling their natural environment. The harmful effects of water pollutants on some organisms may be long delayed and may depend on a complex of environmental factors. Toxicity of the medium may not become apparent until critical life stages of the organisms are reached. Thus, while mayfly nymphs apparently were not affected at certain waste concentrations, the adults failed to emerge as did those in a control stream; and young crayfish appeared to be most sensitive when shedding their exoskeletons in the growth process.

Although the influence of various dilutions of wastes on important life processes of many different aquatic organisms can be conveniently studied in artificial streams, some life processes cannot be so studied, because not every process can be carried out in these streams. Bioassay application factors developed through experiments with artificial streams need verification in the field, where all life processes can be completed.

BENTHIC "SLIME" PRODUCTION, ITS PREDICTION AND PERMISSIBLE LEVELS

The possible interaction of benthic "slimes" with other already critical pollutional factors, resulting in intolerable environmental conditions for some aquatic organisms, has already been discussed in connection with artificial streams. Even where toxicity is not demonstrable and dissolved oxygen appears to be adequate, pollutional "slimes" doubtless can have disastrous effects on stream communities for many streams have a rich fauna dwelling among the rocks of the stream bottom, and this environment can be destroyed by a blanket of "slime" (Tarzwell and Gaufin, 1953). Artificial stream studies have indicated that, with both kraft and calcium base sulfite wastes, benthic "slime" production may possibly be destructive to stream bottom communities at waste concentrations which are not highly toxic nor severly oxygen depleting. Spent sulfite liquor in conjunction with nitrogen-rich domestic sewage in the lower Columbia River has resulted in the production of Sphaerotilus natans to the extent that it interferes with the operation of the commercial gill net fishery there (Amberg and Elder, 1956). Thus the production of benthic "slimes", consisting of Sphaerotilus natans and other bacteria and also various attached algae and protozoa, resulting from the disposal of pulp mill and other organic wastes, may constitute a pollutional problem apart from any other effects of these wastes. Toward the definition and solution of this problem must be directed efforts similar to those which have been applied to problems of oxygen depletion and toxicity in recent years.

The conditions leading to problems of benthic "slime" production may not be the same with different kraft or sulfite process pulp mill wastes and different receiving waters. "Slime" production sufficient to be considered a problem in one area might not be considered a problem in another area. Suitable current conditions and the presence in the receiving water of sufficient nitrogen, phosphorus, and other nutrients are necessary if the wood sugars of the pulp mill wastes are to be fully utilized in "slime" production. Because of these and other variables in the receiving water and the differences in the pulp mill wastes, one mill may have a continual or season "slime" problem while another mill having no greater dilution of its wastes has none.

The level of "slime" production in a receiving water can be determined by observation, even as dissolved oxygen can be determined chemically. Thus, the effectiveness of pulp mill's "slime" production abatement program can be ascertained more directly and easily than the effectiveness of its toxicity control program. But the problem of determining permissible levels of "slime" production for a receiving water is little different from the problem of determining permissible levels of concentration of toxicants, for the levels of "slime" production which do not interfere with any uses of the receiving water must be determined. When it is the protection of aquatic organisms of recreational or commercial value that is of primary importance, the permissible level of "slime" production is that level which in no way decreases the productivity of the valuable aquatic organisms. Thus, the effects of "slime" production on all stages of their life histories, and on the organisms on which they depend for food, must be studied. Many of these effects can be studied in artificial streams

where mortalities or loss of habitat through the "slime" blanketing can be observed. Certain aspects of reproduction and growth of the important organisms will, of necessiry, require study in their natural environments.

Much remains to be learned about the conditions in receiving water which lead to "slime" production and about the "slime" producing potential of various organic wastes. Studies of the environmental requirements of various "slime" organisms and studies of the "slime" producing potential of various kraft and sulfite pulp mill wastes need not all be performed using apparatus as large as the artificial streams discussed earlier, nor need the individual tests last for periods as long as a month. Water quality, temperature, and current velocity, as well as the concentrations of wastes and other principal nutrient substances, are believed to be important factors determining "slime" production, and thus some provision must be made for the control of these in any satisfactory apparatus. For studies of the influence of intermittent discharges of spent sulfite liquor on the production of Sphaerotilus natans, Amberg and Alder (1956) used 2½ by 60 inch Pyrex glass pipes through which water was circulated by small centrifugal pumps to create currents. The circulated water was continually renewed by introducing a constant flow of water with the desired amount of waste added by means of a chemical pump. Each glass pipe contained a plasterer's lath serving as an attachment surface for the "slime". The experiments were of seven-day duration. Modifications of this apparatus may be more suitable for different studies of "slime" production. Small parallel troughs, each receiving constant flows of waste and water and each having a small pump for circulation, would be more convenient for frequent removal of "slime" growth samples. Experience has shown that in such apparatus the accumulated "slime" growth may reach a maximum

after approximately one week. Thus, experiments on "slime" requirements and on the "slime" producing potentialities of various wastes may require at least a week's duration. To facilitate experimentation and more rapid prediction of "slime" production, a standard seeded slide perhaps can be produced at low waste concentrations, thus shortening the time necessary for achieving a measurable or maximal "slime" accumulation when the slide is exposed to the waste concentrations.

When permissible levels of "slime" production in a receiving stream have been established, it may become desirable to control the rate of discharge of waste on the basis of its "slime" production potential in its particular receiving water. A short-term bioassay of this potential of the waste when diluted with its receiving water conceivable could be useful if the relationship between the bioassay result and the permissible level of "slime" production in the receiving water can be expressed as a simple application factor. However, inasmuch as a number of environmental variables other than water quality (e.g., current velocity) greatly influence "slime" production and probably also its biological effects, it is doubtful that such a factor can be recommended for general application. A different application factor may have to be proposed for every different type of stream.

There are reasons for believing that the biochemical oxygen demand may be a fairly satisfactory index of the relative "slime" producing capacity of pulp mill wastes of a given kind in a particular receiving water. Thus, a simple biochemical oxygen demand determination may prove to be usable in conjunction with, or in place of, bioassays involving the actual measurement of "slime" production in controlling waste discharges. The relation between

biochemical oxygen demand and the "slime" producing potential of a waste in its receiving water must, of course, first be established.

VERIFICATION AND UTILIZATION OF BIOASSAY APPLICATION FACTORS

While bioassay application factors developed through the use of artificial streams and other laboratory experimentation may provide adequate protection against damage by pulp mill effluents to those life processes which can proceed normally in the laboratory, important life processes which cannot be completed normally even in unpolluted artificial streams cannot be assumed to be adequately protected. Mortality due to chronic toxicity or to suffocation caused by lack of oxygen or interference with normal respiration, loss of habitat due to "slime" blanketing the benthic environment, and retardation of growth occurring even when adequate food is provided, can all be studied in artificial streams, for the biological processes affected can all take place in such streams. Accordingly, application factors developed by studying these streams might be expected adequately to protect stream organisms against these potential adverse effects of wastes. However, limited survival and growth of valuable organisms does not ensure their continued availability for recreational or commercial purposes. These organisms are valuable because of their ability to replace themselves as they are harvested or as they die of natural causes. Loss of this replaceability, or of their productivity, destroys their value for man, and yet, their productivity is more easily destroyed than is the population itself, for the reproductive processes, the early development, and the complex food chains on which they depend are outstandingly susceptible to derangement by unfavorable environmental conditions.

The embryonic development and the subsequent survival and growth of many organisms can be studied under laboratory conditions. However, some organisms are very difficult to breed in captivity, and the life cycles of the larger forms cannot often be completed in the laboratory, or in the course of experiments of limited duration. Yet, interruption of the natural reproductive cycle at any stage can be disastrous to the population. Further, growth in nature is dependent upon the availability of suitable and sufficient food. While growth can be studied in artificial streams, these streams cannot themselves produce sufficient natural food for normal growth of the larger organisms such as fish. For these reasons, bioassay application factors developed through laboratory studies only cannot be assumed adequately to protect the biological productivity of a receiving water.

To establish that a given waste concentration will not affect the total productivity of a receiving water, it must be demonstrated that the productivities of the plants, the herbivorous and carnivorous invertebrates, and the desirable fish species will not be individually affected. This can, perhaps, best be demonstrated by studying a small stream having a fairly constant rate of discharge, wherein a waste concentration can be experimentally maintained and controlled over long periods. In such a stream, detailed studies of the influence of waste addition and of resulting ecological changes on the growth and reproduction of various groups of organisms can be conducted.

Since artificial streams cannot provide the final answer as to the adequacy of an application factor, it might be suggested that they be dispensed with, using only natural streams instead. However, studies of natural streams would be costly and time-consuming, much time and effort being required for determining all the effects of even a single controlled waste

concentration on the stream biota. Furthermore, some of the factors producing these effects cannot be studied in natural streams in such detail as in artificial streams, which can be modified at will. Therefore, the use of artificial streams as a preliminary step for determining approximate or tentative application factors which are to be further tested through experiments on a natural stream appears to be justified.

Even the small natural stream used experimentally must be regarded only as a "pilot model" of larger streams receiving pulp mill effluents, and studies in small streams are suitable for varifying only in a preliminary way the reliability of bioassay application factors developed through the use of artificial streams. Since receiving waters and pulp mills differ greatly, the adequacy of the application factors for proper control of the discharge of a particular waste must be determined finally through experience and careful observation of the condition of the receiving water and its biota over a long period.

It has been pointed out that the discharge of pulp mill wastes can result in toxic effects on stream inhabitants, depletion of dissolved oxygen in the receiving water, "slime" production, and other undesirable effects. These pollutional effects can occur simultaneously and they can interact, but a single, dominant effect may persist even when the concentration of a waste in the receiving water has been reduced to a level at which the other undesirable effects are no longer demonstrable. The property or characteristic of a waste to which this dominant pollutional effect is referable (e.g., toxicity, oxygen demand, etc.) then can be referred to as the "discharge limiting characteristic" of that waste. Experience has shown

that the discharge limiting characteristic of a particular kind of pulp mill waste (e.g., kraft mill waste) is not always the same, varying from mill to mill and depending on the properties of the receiving water. Often only one kind of bioassay must be performed routinely to measure the discharge limiting characteristic. However, in view of seasonal and other variations of natural stream conditions (temperature, water quality, reaeration rates, etc.), the continued applicability of this particular bioassay first must be thoroughly verified, considering every possible adverse effect of the waste.

Generally speaking, pollutional interference with stream uses by untreated wastes can be avoided in two ways, each of which has its advantages and disadvantages. The first of these involves locating mills only where the minimum stream flows are adequate for assimilation of the maximum amounts of wastes to be produced, so that there is no damage to stream use. The second involves providing facilities for the retention or storage of the wastes and their controlled discharge depending upon the discharge rates and assimilative capacities of the receiving streams. The latter method (controlled dilution) makes possible more complete utilization of the assimilative capacity of receiving waters, and is especially useful where stream discharge rates are subject to great seasonal or other fluctuations. However, this method necessitates waste storage facilities, which cannot always be easily and safely provided, and also the continual regulation of waste discharges in accordance with stream discharge rates and the results of frequently performed bioassays of waste samples. In any case, when the location and waste disposal facilities of a proposed new mill

are planned, appropriate bioassays of mill effluents believed to be similar to those to be discharged from the new mill, together with reliable application factors, can be very instructive and helpful. However, unless proper provision is made for rapid mixing of discharged wastes with the water available for dilution, and unless the possible additive or interacting pollutional effects of other wastes discharged into the water are taken into account, the suggested waste disposal control procedures may prove ineffective.

SUMMARY

Use of the common guppy as a test animal has made possible convenient and highly reproducible acute toxicity bioassays which are deemed essential to the determination of concentrations of pulp and paper mill wastes which will not decrease the productivity of valuable aquatic organisms in receiving waters. Since aquatic organisms cannot maintain themselves normally in their natural environment under conditions which they can barely tolerate in a short-term bioassay, it has been necessary to develop a procedure for establishing the relationship between the results of such a bioassay and permissible waste concentrations in the receiving water. Toxicity bioassay "application factors" are being determined through long-term experiments with aquatic organisms in "artificial streams" into which are introduced known dilutions of pulp and paper mill wastes having predetermined toxicities. Such an "application factor", when applied to the results of an acute toxicity bioassay of a particular waste, presumably can yield a reliable estimate of the waste concentration which will not seriously damage valuable aquatic organisms in the receiving water. Pulp and paper mill wastes may not only be toxic, but may also result in depletion of dissolved oxygen in the

receiving water, or excessive production of bacterial and algal "slime" on the stream bottom. Methods are being developed for studying the "slime" producing potential of wastes; and the relative pollutional importance of each of the changes in the receiving water caused by wastes is being determined.

Conditions caused by waste introduction which are compatible only with the mere survival of some individuals of a once valuable aquatic population cannot be deemed permissible. These aquatic organisms are of commercial or recreational importance because of their capacity to reproduce and grow, continually replacing those harvested by man. This self-replacement capacity, or "productivity", is essentially what must be protected by waste discharge controls.

The complexity of the productivity relationships of aquatic organisms in a natural environment is such that all aspects cannot be studied in artificial streams. Thus, an application factor developed by means of artificial streams must be verified through observations on a small natural stream in which the presumably harmless waste concentration to be tested is maintained over a long period. If the productivity of the valuable organisms under consideration is not influenced at the tested concentration by toxicity, by "slime" growth blanketing the stream bottom, by depressed concentrations of dissolved oxygen, or by other effects of the waste, the application factor used in arriving at this concentration then may be used in computing the permissible rate of discharge of a particular mill effluent into its receiving stream. Differences in receiving waters, wastes, and biological populations necessitate continual examination of the valuable aquatic populations in streams receiving pulp mill effluents, in order to check the adequacy of waste dilution, even when the use of application factors developed by the suggested procedure indicates that no greater dilution is required.

REFERENCES

Amberg, H. R., and R. Elder, 1956. Intermittent discharge of spent sulfite liquor. Jour. Sanitary Engineering Division, Proc. Amer. Soc. Civil Engineers, Proc. Paper 929. Reprinted by Engineering Exper. Station, Oregon State College.

Axelrod, H. R., and L. P. Schultz, 1955. Handbook of tropical aquarium fishes. McGraw-Hill, New York.

Cole, A. E., 1935. Water pollution studies in Wisconsin. Effects of industrial (pulp and paper mill) wastes on fish. Sewage Works Jour., 7: 280-302.

Doudoroff, P., B. G. Anderson, G. E. Burdick, P. S. Galtsoff, W. B. Hart, R. Patrick, E. R. Strong, E. W. Surber, and W. M. Van Horn, 1951. Bio-assay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes, 23: 1380-1397.

Ebeling, G., 1931. Neuere Ergebnisse bei der fischereilich chemischen Untersuchungen und Beurteilung von Zellstoffabrikabwassern. Vom Wasser, 5: 192-200.

Ebeling, G., 1932. Untersuchungen über die Wirkung von Zellstoffabrikabwassern auf Fische und Fischnahrtiere. Zeitschrift für Fischerei, 30: 341-410.

Hart, W. B., P. Doudoroff, and J. Greenbank, 1945. The evaluation of the toxicity of industrial wastes, chemicals and other substances to freshwater fishes. Waste Control Lab., Atlantic Refining Co., Philadelphia.

Haydu, E. P., H. R. Amberg, and R. E. Dimick. The effect of kraft mill waste components on certain salmonoid fishes of the Pacific Northwest. Tappi, 35: 545-549.

Jones, B. F., C. E. Warren, C. E. Bond, and P. Doudoroff, 1956. Avoidance reactions of salmonid fishes to pulp mill effluents. Sewage and Industrial Wastes, 28: 1403-1413.

McHugh, R. A., 1954. Preliminary report on a study of the factors responsible for the toxicity of wastes from a modern kraft pulp and paper mill. Unpublished Report, Department of Fish and Game Management, Oregon State College, Corvallis, Oregon.

Tarzwell, C. M., and A. R. Gaufin, 1953. Some important biological effects of pollution often disregarded in stream surveys. Proc. 8th Indus. Waste Conf., Purdue Univ., Eng. Bull. Series, 83: 295-316.

Van Horn, W. M., J. B. Anderson, and M. Katz, 1950. The effect of kraft pulp mill wastes on some aquatic organisms. Trans. Amer. Fisheries Soc. (for 1949), 79: 55-63.

Warren, C. E., and P. Doudoroff, 1957. Cooperative research at Oregon State College in the biological aspects of water pollution. Biological Problems in Water Pollution, Trans. Seminar on Biological Problems of Water Pollution (1956), R. A. Taft Sanitary Engineering Center, U. S. Public Health Service, Cincinnati, Ohio.